REMARKS

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein is respectfully requested. Claims 1 and 5-6 are amended. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the prosecution of the claims prior to amendment, which claims are present in a continuation of the above-referenced application. Claims 1-67 are now pending in this application.

The 35 U.S.C. § 112 Rejections

Claims 4-6 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The amendments to claims 5 and 6 obviate the rejection, as the recited restriction enzymes generate blunt ends.

Thus, withdrawal of the § 112(2) rejection is respectfully requested.

Claims 10-12 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. This rejection is respectfully traversed.

The Examiner is respectfully reminded that Applicant need not teach what is well-known to the art. With regard to "X_1-X_3, X_2X_3G or X_3GC is a codon which is not a stop codon" in claim 10, "$X_1X_2X_3$ is a codon in an open reading frame which is not a stop codon" in claim 11, and "$X_1X_2X_3$, X_2X_3G or X_3GT is a codon in an open reading frame which is not a stop codon" in claim 12, the Examiner is requested to consider pages 900-901 in Metzler: Biochemistry: the Chemical Reactions of Living Cells, Academic Press, Inc. (1977) (a copy is enclosed herewith), where every codon including stop codons is described.

Moreover, Applicant has described exemplary restriction enzymes that 1) generate a 3' TA overhang, 2) generate blunt ends, and 3) have infrequent sites in cDNAs and generate blunt ends (see, for example, pages 16-17 and 49-50 of the specification). Accordingly, one of skill in the art in possession of Applicant's specification would be apprised that Applicant was in possession of the claimed vectors.

Therefore, withdrawal of the § 112(1) "written description" rejection is respectfully requested.

The 35 U.S.C. § 102(b) Rejection

Claims 1-8 were rejected under 35 U.S.C. § 102(b) as being anticipated by Bilcock et al. (J. Biol. Chem., 274:36379 (1999)). This rejection, as it may be maintained with respect to the pending claims, is respectfully traversed.

Bilcock et al. disclose 5 plasmids (Figure 1) with a plurality of restriction endonuclease recognition sites designed to determine whether certain Type II enzymes that recognize a site with 8 specified base pairs require two sites per molecule or can cleave a molecule with only one site.

pAT153, pDB7, and pDB8 in Figure 1 of Bilcock et al., if cleaved with a restriction enzyme that generates a 3' TA overhang (*Sgf*I in pAT153, pDB7, and pDB8) and a restriction enzyme which generates blunt ends (*Srf*I in pAT153, pDB7, and pDB8), would not yield a vector backbone where the end generated by *Sgf*I could be ligated 5' to an open reading frame, because the restriction enzyme which generates blunt ends in pAT153, pDB7, and pDB8 is 5' to the site for *Sgf*I.

Moreover, pNEB193 and pAB1 (pAB1 is derived from pNEB193) in Figure 1 of Bilcock et al., if cleaved with a restriction enzyme that generates a 3' TA overhang (*Pac*I in pNEB193 and pAB1) and *Ssp*I or *Pvu*II in pNEB193 or *Pme*I in pAB1, would not yield a vector backbone where the end generated by *Pac*I is 5' to the open reading frame. That is because *Ssp*I in pNEB193 is 5' to *Pac*I, and *Pvu*II in pNEB193 and *Pme*I in pAB1 have two sites that flank *Pac*I.

Further, pNEB193 and pAB1 do not include a promoter operably linked to an open reading 5' to *Pac*I (see enclosed summary of genetic elements in pNEB193).

Therefore, withdrawal of the § 102(b) rejection is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance, and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney at (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date January 18, 2007

By Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Mail Stop Amendment, Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 18th day of January 2007.

Dawn M. Poole

Name

Signature

BIOCHEMISTRY

The Chemical Reactions of Living Cells

DAVID E. METZLER

ferred from the donor strain. Using this technique it was found that complete transfer of the chromosome takes $\sim$100 min at 37°C and that the *approximate location of any gene on the chromosome can be determined by the length of time required for transfer of that gene into the recipient cell.* It is a little more complex than this. Because complete chromosome transfer is rare, substrains of *E. coli* K-12 with the F agent integrated at different points are used. In each case, those genes lying clockwise* around the circle in Fig. 15-1 immediately beyond the point of integration are transferred quickly and with high frequency.

The map in Fig. 15-1 is based not only on interrupted matings but also on the use of "transduction" by bacteriophage P1.[15] Transduction by phage, discussed in more detail in Section D, permits the transfer of a short fragment of DNA, about 2 min in length, on the *E. coli* map. Joint transduction, i.e., joint incorporation of two genes into the chromosome of the receptor, occurs with a frequency related to the map distance between these two genes. Thus, finer mapping has been done within many segments of the *E. coli* chromosome.

Note that while the map in Fig. 15-1 is calibrated in minutes, this is regarded as a temporary expedient. It will soon be possible to express the linkage map directly as micrometers of DNA length (total length $\sim$1100 μm) or in thousands of nucleotide units, sometimes called **kilobases** (kb). The total length is $\sim$3800 kb.†

2. The Genetic Code

The general nature of the genetic code was suggested by the structure of DNA itself. Both DNA and proteins are linear polymers. Thus, it seemed logical to suppose that the sequence of the bases in DNA coded for the sequence of amino acids. Since there are only four bases in DNA but 20 different amino acids in proteins (at the time of their synthesis) each amino acid must be specified by some combination of more than one base. While 16 *pairs* of bases are possible, this is still too few to specify 20 different amino acids. Therefore, it appeared that at least a *triplet* group of three nucleotides would be required to code for one amino acid.[16] Sixty-four (4^2) such triplet **codons** exist, as is indicated in Tables 15-2 and 15-3.

TABLE 15-2
The Genetic Code[a]

Amino acid	Codons	Total number of codons
Alanine	GCX	4
Arginine	CGX, AGA, AGG	6
Asparagine	AAU, AAC	2
Aspartic acid	GAU, GAC	2
Cysteine	UGU, UGC	2
Glutamic acid	GAA, GAG	2
Glutamine	CAA, CAG	2
Glycine	GGX	4
Histidine	CAU, CAG C	2
Isoleucine	AUU, AUC, AUA	3
Leucine	UUA, UUG, CUX	6
Lysine	AAA, A~~U~~G A	2
Methionine (also initiation codon)	AUG	1
Phenylalanine	UUU, UUC	2
Proline	CCX	4
Serine	UCX, AGU, AGC	6
Threonine	ACX	4
Tryptophan	UGG	1
Tyrosine	UAU, UAC	2
Valine (GUG is sometimes an initiation codon)	GUX	4
Termination	UAA (*ochre*) UAG (*amber*) UGA	3
Total		64

[a] The codons for each amino acid are given in terms of the sequence of bases in messenger RNA. From left to right the sequence is from the 5′ end to the 3′ end. The symbol X stands for any one of the four RNA bases. Thus each codon symbol containing X represents a group of four codons.

* With one type of F factor. Others are integrated in the opposite direction.

† This value is somewhat uncertain. Thus the map in Fig. 15-1 is based[15] on a total length of 4100 kb or a molecular weight of 2.7 × 10⁹.

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TABLE 15-3
The Sixty-Four Codons of the Genetic Code

5'-OH Terminal base	Middle base U(T)	C	A	G	3'-OH Terminal base
U(T)	Phe	Ser	Tyr	Cys	U(T)
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term	Term	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met[a]	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val[a]	Ala	Glu	Gly	G

[a] Initiation codons. The methionine codon AUG is the most common starting point for translation of a genetic message but GUG can also serve. In such cases it codes for methionine rather than valine.

Simplicity argued that the genetic blueprint specifying amino acid sequences in proteins should consist of *consecutive, nonoverlapping triplets*. However, there was initially no proof of this and other possibilities were actively considered, but within a few years genetic experiments (some of which are discussed in Section D) together with chemical experiments considered in the following section provided unequivocal proof for a nonoverlapping code.

Deciphering the Code

Even after the triplet nature of the genetic code became evident, many questions remained. Were all of the 64 possible codons used by the living cell? If so, were they all used to code for amino acids or were some set aside for other purposes? How many codons were used for a single amino acid? Was the code "universal," applying to all organisms, or did different organisms use different codes? How could one decipher the code? Despite the complexity of these questions, they all seem to have been answered definitively.

An important experiment[17] was performed by M. Nirenberg* and H. Matthaei in 1961. Using a typical biochemist's approach, Nirenberg had isolated ribosomes from *E. coli*. He mixed these with crude extracts of soluble materials, also from *E. coli* cells. The extracts included tRNA molecules and amino acid activating enzymes. The 20 amino acids, ATP, and an ATP-generating system (PEP + pyruvate kinase) were added. Nirenberg was able to show that under such conditions protein was synthesized by ribosomes in response to the presence of added RNA. For example, RNA from tobacco mosaic virus (Chapter 4, Section D,2) was very effective in stimulating protein synthesis. The crucial experiment (originally done simply as a "control") was one in which a synthetic polynucleotide consisting solely of uridylic acid units was substituted for mRNA. In effect, this was a synthetic mRNA containing only the codon UUU repeated over and over. To Nirenberg's surprise, the ribosomes read this code and synthesized a peptide containing only phenylalanine. Thus, poly(U) gave polyphenylalanine and *UUU was identified as a codon specifying phenylalanine.* The first nucleotide triplet had been identified! In the same manner CCC was identified as a proline codon and AAA as a lysine codon. Study of mixed copolymers containing two different nucleotides in a random sequence suggested other codon assignments. However, it was a few years later, after H. G. Khorana had supplied the methods for synthesis of oligonucleotides and of regular alternating polymers of known sequence, that the remaining codons were identified.

An important technique was based on the observation that synthetic trinucleotides induced the binding to ribosomes of specific tRNA molecules "charged" with their specific amino acids.[18,19] For example, the trinucleotides UpUpU and ApApA stimulated the binding of ^{14}C-labeled phenyl-

* In 1968 Nirenberg and Khorana together with R. Holley, who first determined the sequence of a transfer RNA, were awarded a Nobel Prize.

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!!NA_SEQUENCE 1.0
Plasmid pNEB193

Update 6/11/03

Features:
   496- 146      lacZ alpha CDS (start 496, complementary strand)
   546- 541      Plac promoter -10 sequence (TATGTT)
   570- 565      Plac promoter -35 sequence (TTTACA)
   602- 590      CAP protein binding site
   396- 479      multiple cloning site (EcoRI-HindIII)
  1482- 894      origin of replication (counterclockwise)
                   (RNAII -35 to RNA/DNA switch point):
  1300-1305        RNAI transcript promoter -35 sequence (TTGAAG)
  1322-1327        RNAI transcript promoter -10 sequence (GCTACA)
  1336-1443        RNAI transcript
  1446- 894        RNAII transcript (complementary strand)
  1461-1456        RNAII transcript promoter -10 sequence (CGTAAT)
  1482-1477        RNAII transcript promoter -35 sequence (TTGAGA)
  2513-1653      beta-lactamase (bla; amp-r) CDS
                   (start 2513, complementary strand)
  2513-2445      beta-lactamase signal peptide CDS
                   (start 2513, complementary strand)

pneb193.seq  Length: 2713  June 11, 2003 12:46  Type: N  Check: 1526
..

       1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG

      51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG

     101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG

     151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA

     201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC ATTCGCCATT

     251  CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT

     301  TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA

     351  ACGCCAGGGT TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT

     401  CGAGCTCGGT ACCCGGGGGC GCGCCGGATC CTTAATTAAG TCTAGAGTCG

     451  ACTGTTTAAA CCTGCAGGCA TGCAAGCTTG GCGTAATCAT GGTCATAGCT

     501  GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC AACATACGAG

     551  CCGGAAGCAT AAAGTGTAAA GCCTGGGGTG CCTAATGAGT GAGCTAACTC

     601  ACATTAATTG CGTTGCGCTC ACTGCCCGCT TTCCAGTCGG GAAACCTGTC

     651  GTGCCAGCTG CATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGC

     701  GTATTGGGCG CTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC
```

751 GTTCGGCTGC GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT

801 ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC

851 AGCAAAAGGC CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT

901 AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG

951 GTGGCGAAAC CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA

1001 GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG

1051 TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG

1101 TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC

1151 ACGAACCCCC CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT

1201 CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC

1251 TGGTAACAGG ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT

1301 TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGAACAGT ATTTGGTATC

1351 TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG

1401 ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC

1451 AGCAGATTAC GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT

1501 TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT

1551 GGTCATGAGA TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA

1601 AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC

1651 AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT

1701 TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC

1751 GGGAGGGCTT ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA

1801 CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC

1851 CGAGCGCAGA AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA

1901 ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC

1951 AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG

2001 TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT

2051 CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT

2101 GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT

2151 GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG

2201 GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT

2251 TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC

2301 TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA

2351 GGATCTTACC GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC

2401 AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA

2451 AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT

2501 GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG

2551 GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA

2601 ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT

2651 AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG

2701 AGGCCCTTTC GTC